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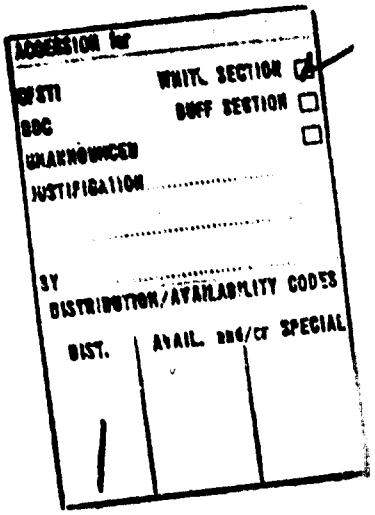

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FOREWORD

This report was prepared in the Radiobiology Division, under task No. 775703. The work was accomplished between November 1965 and March 1968, and the paper was received for publication on 18 July 1968.

R. S. Martinez prepared some of the preliminary data used in the study.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
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ABSTRACT

To determine whether a combination of derivatives causes less toxicity in achieving the radioprotective effect of tryptamine, laboratory rats were injected intraperitoneally with 5-hydroxytryptamine alone, and in combination with 5-methoxytryptamine and sodium hexobarbital, before exposure to 1,200 R gamma radiation. Results show that survival increases when the agents are given in certain combinations. Thirty-day survival (27/30) of rats treated with the triple drug combination was significantly greater than the survival of rats treated with any of the drugs individually. These data suggest that an additive or synergistic effect may be present when the agents are administered in combination. The data also suggest that radioprotection by indolylalkylamines is more effective against 1,200 R gamma delivered over a short interval than against the same dosage over a more extended interval. The optimum time for drug administration was found to be approximately 15 minutes before irradiation. To extend this radioprotection to primates, *Macaca mulatta* were injected intraperitoneally with the triple drug combination before exposure to 850 R x-rays. Of the treated primates, 4 out of 15 survived.

INDOLYLALKYLAMINES AS RADIOPROTECTANTS

I. INTRODUCTION

Chemical radioprotectants have been recognized as partially effective by many investigators (3, 4, 7). The toxicity of most of these agents constitutes a common problem to biologic systems and requires careful evaluation (14). In administering a chemical radioprotectant, care must be taken to maintain the safe maximum dosage, to use the least traumatic route of medication, and to compound the agents in a form for rapid distribution and metabolism. The problem is further complicated when drugs or chemicals are combined in a biologic system. Here, one often encounters the unexpected results of either drug antagonism or drug synergism (5, 6).

This study was prompted by our findings that pentobarbital will potentiate the action of sulfhydrylamines such as AET, as well as tryptamines such as serotonin, in rodents (13), and that 5-methoxytryptamine is less toxic and better tolerated than 5-hydroxytryptamine in rodents (12). Therefore a mixture of the two tryptamines might be expected to afford less toxicity for a given concentration of tryptamine, and the radioprotective effect might be potentiated by the appropriate addition of a barbiturate-type drug (13).

II. METHOD

Animal care and maintenance

Male Sprague-Dawley rats were obtained from a Berkeley, Calif., laboratory. These albino rats averaged approximately 200 gm. in weight at the time of arrival. After 10 days of quarantine in the vivarium they were transferred to wire-mesh colony cages in an air-conditioned room. They were fed Purina laboratory chow and water ad libitum. The rats were ear-tagged, weighed, and randomly placed in groups of 6. Each group occupied a separate compartment in the colony cage. The rate of weight gain observed during this regimen was normal according to sales literature furnished by the supplier.

Primates *(Macaca mulatta)* used in this experiment were Asiatic imports. They had been clinically examined and quarantined before delivery to our facility. Upon arrival, they were again quarantined for 30 days and standardized for our experiment. Standardization included fecal examination for ova and parasites, bacterial culturing for pathogenic flora, tuberculosis screening, and physical examination for gross abnormalities.

Reagents

Our stock component medications were obtained from commercial sources, and the drug preparations were compounded in our laboratory just before use. These combinations were dissolved in sterile distilled water. The drug solutions were then passed through a sterile Millipore filter. Injected doses were based on individual animal weight and measured into tuberculin syringes. All injections were made intraperitoneally. No rat received more than 1 cc. of solution.

The 5-hydroxytryptamine (5-HT) referred to throughout this report is the 5-hydroxytryptamine creatinine sulfate complex; its molecular weight is 405.4. The 5-methoxytryptamine (5-MT) has a molecular weight of 190.2 and a melting point of 120 C.

Radiation

Gamma radiation from a 5,000 c. ^{60}Co source (8) was delivered to groups of 6 rats held in six individual compartments within a horizontal, revolving, wire cylinder. Preliminary irradiations were done in a stationary, rectangular wire cage having individual rat compartments. Dose rates varied from 74 R/min. to 800 R/min. Measurements were calculated from a ^{60}Co source decay chart and also by ion chamber dosimeters (Victoreen, 100 R). Variations in dose measurements of less than 3% total dosage were considered as being identical.

X-irradiation was delivered from a Maxitron 300 machine. The 300 kvp therapy machine was operated at a current of 20 ma. Filtration was acomplished with a standard Al-Cu-Sn filter which yields a half-value layer (HVL) of 2 mm. copper. The target-subject-distance (TSD) was 135 cm. from the point of delivery to the midline of the primate held in a vertical, revolving wire-mesh cylinder. Doses were measured by ion chamber dosimeters (Victoreen, 100 R), and the x-ray beam was constantly monitored with a Victoreen rate meter, reading from a probe located adjacent to the midline of the rotating primate.

III. RESULTS

A modified Dixon and Mood method, as reported by Kimball et al. (9), was employed to establish the toxicity (LD_{50}) and the safe maximum dosages of the drugs used. This method yields results within a 5% S.E. range. Preliminary studies (12) established the LD_{50} of the agents to be as shown in table I. The LD_{50} of 5-HT was 39 mg./kg. body weight when given intraperitoneally to rats, as compared with 80 mg./kg. for 5-MT, and 441 mg./kg. for sodium hexobarbital. These results established 5-HT as the limiting component in our combinations.

Results of preliminary radioprotection studies of each drug administered separately are shown in table II. A study conducted approximately one year later confirmed the radioprotective capacity of selected dose levels as shown in table III. In both instances, sodium hexobarbital alone yielded negligible radioprotection, and 5-hydroxytryptamine, in the amount of 10 mg./kg., appeared to be twice as effective as the same amount of 5-MT. This finding compares favorably with earlier data in the literature (1, 4, 17).

TABLE I

Results of preliminary drug toxicity studies

Drug	LD_{50} (mg./kg.)
Sodium hexobarbital	441
5-HT	39
5-MT	80

Animals used: Sprague-Dawley rats.
Method of injection: Intraperitoneal.
Time observed: 24 hours.

TABLE II

Efficacy of three drugs by dosage and injection time

Drug and dosage	Survival ratio*		
	10 min.†	20 min.†	40 min.†
Sodium hexobarbital (mg./kg.)			
10	0/6	0/6	0/6
20	0/6	0/6	0/6
40	0/6	0/6	0/6
100	0/6	0/6	2/6
200	0/6	0/6	1/6
300	0/6	1/6	0/6
5-HT (mg./kg.)			
10	5/5	2/5	1/5
20	2/5	2/5	5/5
35	1/5	2/5	0/5
5-MT (mg./kg.)			
15	3/6	1/6	0/6
30	2/6	4/6	1/6
60	1/6	3/6	4/6

*Rat survival at 30 days against 1,200 R gamma irradiation.
†Injection time before irradiation.

Several different drug combinations were tested for drug toxicity. The best combination appeared to be that of 10 mg./kg. 5-HT, 20 mg./kg. 5-MT, and 10 mg./kg. sodium hexobarbital, which yielded no drug toxicity deaths. Three higher concentrations were tried but were discarded because of higher toxicity.

An optimum combination dose, the "10-20-10" combination, was established and this dose was tested for its radioprotective capacity as shown in table IV. The experiment was designed to test also the optimum time for injection prior to irradiation. Cobalt-60 gamma radiation of 1,200 R was selected as the approximate LD_{90} radiation dose for our Sprague-Dawley rats. The previous $LD_{50/30}$ for these rats in our laboratory was 931 R ^{60}Co (12).

In a series of experiments to determine the protection afforded when two or more of the three drugs are combined, we achieved results as shown in table V. Combining 5-HT with sodium hexobarbital yielded a lower (25%) survival than treatment with 5-HT alone. This experiment was repeated and similar results were obtained. Combining 5-MT with sodium hexobarbital yielded 42% survival. When 5-HT and 5-MT were combined, the survival rate increased to 75%. When all three drugs were combined and injected in rats exposed to 1,200 R gamma at 400 R/min., three separate experiments yielded survival of 67%, 70%, and 78%, respectively.

Matched experiments were performed to determine the optimum time for injection and the least lethal rate of administering 1,200 R ^{60}Co. It was found that fewer deaths occurred in groups injected 15 minutes before rather than 10 or 20 minutes before irradiation as seen in table VI. Also, fewer deaths were

TABLE III

Radioprotection afforded by drugs individually

Drug	Survival ratio*
5-HT (10 mg./kg.)	12/24
5-MT (20 mg./kg.)	12/24
Sodium hexobarbital (10 mg./kg.)	0/24

*Rat survival at 30 days against 1,200 R gamma irradiation.

TABLE IV

Optimum "10-20-10" dosage at various injection times (preliminary study, May 1966)*

Before irradiation (min.)	Survival ratio†
10	8/10
20	4/6
30	3/6

*Combined drug dose: 5-HT (10 mg./kg.), 5-MT (20 mg./kg.), and sodium hexobarbital (10 mg./kg.).

†Rat survival at 30 days against 1,200 R gamma irradiation.

TABLE V

Radioprotection afforded by drugs in combination

Drugs	Survival ratio*
5-HT and sodium hexobarbital	6/24
5-MT and sodium hexobarbital	10/24
5-HT and 5-MT	18/24
5-HT, 5-MT, and sodium hexobarbital	
Experiment 1	16/24
Experiment 2	17/24
Experiment 3	14/18

*Rat survival at 30 days against 1,200 R gamma irradiation, at 400 R/min.

TABLE VI

Effect of injection time on efficacy of three drugs in combination

Before irradiation (min.)	Survival ratio*
10	18/36
15	26/36
20	19/36

*Rat survival at 30 days against 1,200 R gamma irradiation. The three drugs used were 5-HT, 5-MT, and sodium hexobarbital, in the "10-20-10" dosage.

observed in groups irradiated at 800 R/min. for 1.5 minutes than in groups irradiated at 200 R/min. for 6 minutes, or 400 R/min. for 3 minutes, as shown in table VII.

In an experiment to demonstrate maximum survival in rats, 30 rats were treated with the triple drug combination 15 minutes before being irradiated with 1,200 R ^{60}Co at 800 R/min. Of the treated rats, 27 out of 30 survived 30 days, while only 1 out of 30 control rats irradiated identically survived. Thus, the maximum survival obtained in these experiments was 90%.

The triple agent combination was given to a group of 45 rats 15 minutes prior to 1,200 R ^{60}Co (administered at 74 R/min. for 16¼ minutes) to establish the degree of protection of the same drug dose at extended periods of radiation. Forty-five untreated control rats were irradiated similarly. In this test, we achieved 33% survival among the treated rats. All untreated rats had died by the end of the tenth day postirradiation.

This radioprotective combination was extended to primates. A group of 15 standardized primates were injected intraperitoneally with 10 mg./kg. of 5-HT, 20 mg./kg. of 5-MT, and 10 mg./kg. of sodium hexobarbital, 15 minutes before exposure to 850 R x-irradiation. A group of 12 standardized primates served as untreated radiation controls and received 850 R x-rays without any medication. Of the treated primates, 2 out of 15 survived

TABLE VII

Effect of dose rate on efficacy of three drugs in combination

Dose rate (R/min.)	Survival ratio*
74	15/45
200	14/36
400	25/36
800	27/30

*Rat survival at 30 days against 1,200 R gamma irradiation. The three drugs used were 5-HT, 5-MT, and sodium hexobarbital, in the "10-20-10" dosage.

30 days and were sacrificed, while all 12 irradiated controls died from the radiation syndrome between the tenth and fifteenth day postirradiation.

An effort to increase survival time was made by increasing the radioprotectant dose of each component by 50%. Both 5-HT and sodium hexobarbital were increased to 15 mg./kg., and 5-MT was increased to 30 mg./kg. In this experiment 4 out of 15 treated primates survived 30 days and no untreated controls survived, as illustrated in table VIII.

IV. DISCUSSION

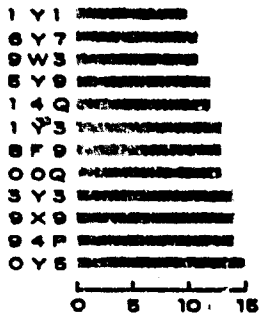
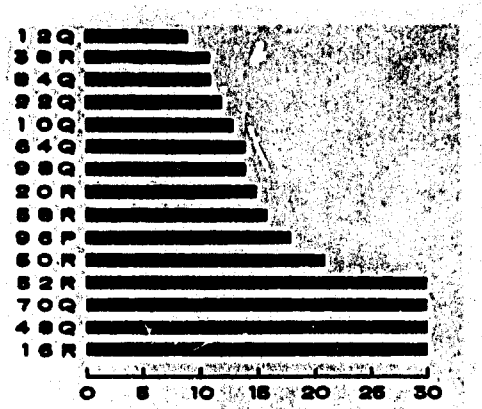
Gray et al. (7) and Bacq and Herve (3) first demonstrated the protective effects of 5-HT against doses of x-irradiation. These investigators concerned themselves primarily with the protection of mice and rats against low energy x-irradiation at doses of 700 to 880 R. More recent data on indolylalkylamines has been contributed by several investigators (10, 11, 15, 16, 17). Our experiments were designed to study the protection of rats against high energy gamma irradiation of 1,200 R, and more significantly, the protection of primates against a superlethal dose of 850 R x-rays. These data afford a comparison between differences in species of mammals and types of ionizing radiation.

Of interest also is the difference in toxicity of 5-HT in rats as compared to the relatively mild toxicity of 5-HT in mice as indicated by Bacq (2). This investigator achieved 100% survival in at least one group of mice by administering 220 mg./kg. of 5-HT as the radioprotectant. The LD_{50} of 5-HT in our rats (39 mg./kg. I.P.) offers an immediate contrast between species.

Although the radioprotective capacity of this drug mixture proved successful in rats against 1,200 R gamma, only limited success was achieved in the primate against 850 R x-rays. An alternate route of administration may improve radioprotection with this drug mixture. Our necropsies revealed that the combination agent, when injected intraperitoneally,

TABLE VIII

Survival of x-irradiated primates treated with three drugs in combination*

Control	Experimental
	

*850 R x-rays.

caused extensive peritonitis in the abdominal wall and the omentum of the treated animals. This condition was not conducive to survival and may have diminished the total number of survivors. Additionally, recent preliminary experiments have shown this same drug mixture can be administered intravenously without detrimental results.

V. CONCLUSIONS

The enhanced radioprotective capacity of mixtures of 5-HT, 5-MT, and sodium hexobarbital in rats exposed to 1,200 R gamma irradiation, as compared to the much lower radioprotection offered by the individual drugs tested identically, can perhaps be best attributed to the effects of drug synergism according to the definitions of Gaddum (5) or Goth (6). Potentiation by sodium hexobarbital has not been shown by these data, although data (12) by other investigators suggest that potentiation by hexobarbital does occur in chemical protection.

The decreased radioprotective capacity of this mixture in primates exposed to 850 R x-rays cannot be easily explained. Protecting the more complex, higher, mammalian system involves both known factors such as species differences and unknown factors which can only be elucidated through more definitive research in this area. The intravenous route of administration might improve the radioprotective capacity of this drug system in primates.

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13. ABSTRACT

To determine whether a combination of derivatives causes less toxicity in achieving the radioprotective effect of tryptamine, laboratory rats were injected intraperitoneally with 5-hydroxytryptamine alone, and in combination with 5-methoxytryptamine and sodium hexobarbital, before exposure to 1,200 R gamma radiation. Results show that survival increases when the agents are given in certain combinations. Thirty-day survival (27/30) of rats treated with the triple drug combination was significantly greater than the survival of rats treated with any of the drugs individually. These data suggest that an additive or synergistic effect may be present when the agents are administered in combination. The data also suggest that radioprotection by indolylalkylamines is more effective against 1,200 R gamma delivered over a short interval than against the same dosage over a more extended interval. The optimum time for drug administration was found to be approximately 15 minutes before irradiation. To extend this radioprotection to primates, Macaca mulatta were injected intraperitoneally with the triple drug combination before exposure to 850 R x-rays. Of the treated primates, 4 out of 15 survived.

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